

# Inhibition of Beta-1 Receptor but not Vagotomy Can Abolish the L-NAME Evoked Bradycardia in Anesthetized Rat

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## Summary

We reported previously that the nitric oxide synthesis inhibitor N<sup>ω</sup>-nitro-L-arginine methyl ester (L-NAME) decreases cardiac output. Several studies have shown that inhibition of nitric oxide synthesis decreases the heart rate. In the present study, we investigated the effect of a single bolus administration of L-NAME on blood pressure and heart rate monitored for one hour in anesthetized rats and the influence of vagotomy and  $\beta_1$ -receptor blocker metoprolol on the L-NAME induced bradycardia. After L-NAME treatment, the blood pressure rose immediately after the injection of the drug (peak response in the third minute: +24 %,  $p < 0.001$ ) and fell to the control level in the 20th minute. The heart rate decreased immediately after L-NAME administration, the lowest value being reached in the 10th minute (-14 %,  $p < 0.001$ ). However, bradycardia was sustained even after the blood pressure had returned to the control level. Bilateral vagotomy failed to influence the negative chronotropic effect of L-NAME, but bradycardia was completely abolished by metoprolol pretreatment. We concluded that the bradycardia evoked by L-NAME is mainly due to the withdrawal of sympathetic tone upon the heart rate. However, the cause of sustained bradycardia after normalization of blood pressure cannot be elucidated.

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## Key words

Nitric oxide • Vagotomy • Metoprolol • Heart rate

## Introduction

The administration of the nitric oxide synthase (NOS) inhibitor increases arterial blood pressure within minutes. Either acute or chronic administration of NOS inhibitor not only elevates arterial blood pressure but also decreases cardiac output (Hennessy *et al.* 1994, Lechevalier *et al.* 1994, Wang *et al.* 1995). In previous experiments, we also observed a decrease of cardiac output after administration of the NOS inhibitor L-NAME (Hably *et al.* 1998, Vág *et al.* 1998). There are

two mechanisms that can reduce the cardiac output: decrease of the contractile force of the cardiac muscle and/or decrease in the heart rate. In several studies, bradycardia was observed after blockade of nitric oxide production evoked by different arginine analogues in various species in conscious or unconscious animals (Du *et al.* 1992, Jones and Brody 1992, Persson *et al.* 1992, Manning *et al.* 1993, Hennessy *et al.* 1994, Reid *et al.* 1994).

The aim of the present study was to investigate the effect of L-NAME on the heart rate and to clarify the

role of parasympathetic (vagus nerve) and sympathetic ( $\beta_1$ -receptor mediated) innervation of the heart in the changes of the heart rate.

## Methods

Female Wistar rats weighing between 160 and 220 g were anesthetized with Nembutal (60 mg/kg b.m., ip.). Rats were placed in the supine position with their head fixed to a heated table. Body temperature was kept at about 37 °C. A catheter was inserted into the right external jugular vein, and the left common carotid artery was cannulated. Tracheostomy was performed and the animals were allowed to breathe spontaneously. In the experiments where vagotomy was performed, bilateral vagal nerves next to the common carotid artery were exposed. After completion of the surgery, heparin (500 IU/kg iv.) was given. Blood pressure was measured in the common carotid artery with a Statham pressure transducer and was monitored (Medicor EM-61 recorder) throughout. In order to calculate the heart rate standard

ECG was recorded. The rats were left undisturbed until heart rate and blood pressure stabilized.

Drugs were administrated as bolus injection through the external jugular vein. Nitric oxide synthesis was blocked by L-NAME (10 mg/kg b.m. in 0.25 ml 0.15 M sodium chloride solution/100g b.m.). The  $\beta_1$ -adrenergic receptors were selectively blocked by metoprolol (15 mg/kg b.m. in 0.375 ml 0.15 M sodium chloride solution /100 g b.m.).

In the first series of experiments, the effect of L-NAME on arterial blood pressure and heart rate was monitored during 60 min. Following stabilization of blood pressure and the heart rate (baseline period), L-NAME was administered (n=12) as described above. Control rats (n=11) were given the same amount of vehicle.

In the second series of experiments the role of parasympathetic (nervus vagus) and sympathetic nerves were investigated in the L-NAME evoked bradycardia. The animals were divided into four groups. After recording control values (baseline) two interventions were made as is shown in Table 1.

**Table 1.**

	<b>Intervention 1</b>	<b>Intervention 2 (10 min after the Intervention 1)</b>
<i>I. group (n=8)</i>	L-NAME administration	Bilateral vagotomy
<i>II. group (n=8)</i>	Bilateral vagotomy	L-NAME administration
<i>III. group (n=10)</i>	Metoprolol administration	L-NAME administration
<i>IV. group (n=10)</i>	Bilateral vagotomy + metoprolol	L-NAME administration

## Statistics

All values are given as means  $\pm$  standard error of the means (S.E.M.). Statistical analysis was performed using Student's paired t-test (alterations in the same group). Repeated measurements of ANOVA were used to calculate interaction between interventions. The P value <0.05 was taken as significant.

## Results

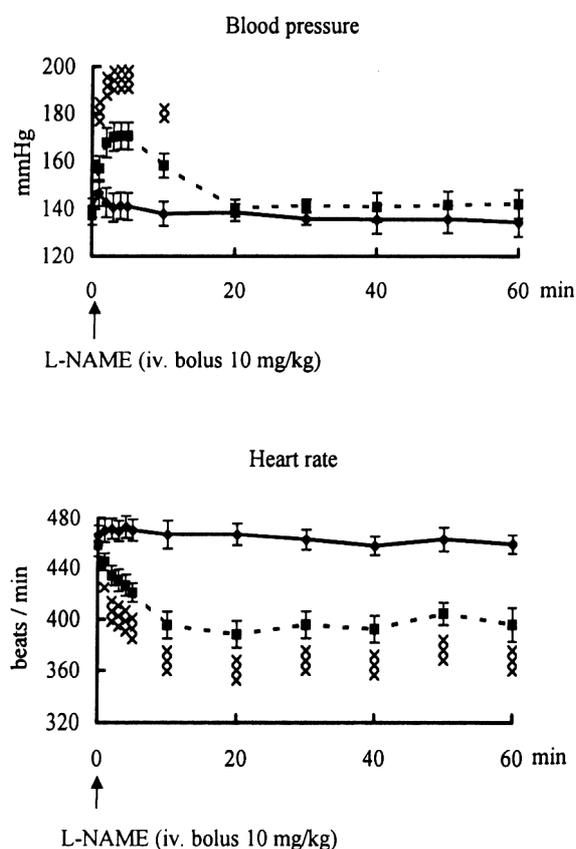
### *Effect of L-NAME on the blood pressure and heart rate - First series of experiments*

In the L-NAME treated rats the blood pressure rose immediately after the injection of the drug (Fig. 1). The peak level was reached in the third minute and it was

24 % higher ( $170 \pm 6.0$  mm Hg) than before L-NAME administration ( $137 \pm 4.0$  mm Hg,  $p < 0.001$ ). Although a moderate decrease of blood pressure could be observed from the fifth minute on, the blood pressure was significantly higher even in the 10th min after the L-NAME administration ( $158 \pm 5.1$  mm Hg,  $p < 0.01$ ), and it was decreased to the control level in the 20th min ( $140 \pm 3.6$  mm Hg). In the control rats (time-control experiment) the blood pressure increased immediately after the injection of the solvent (from  $141 \pm 2.7$  to  $147 \pm 2.9$  mm Hg,  $p < 0.01$ ), but it returned to the starting level within two minutes and it did not change significantly within one hour.

The heart rate in the time-control experiments (control group) remained constant in the course of investigation (Fig. 1). However, in the L-NAME group the heart rate decreased immediately after L-NAME

administration (from  $458 \pm 9.1$  to  $445 \pm 7.0$  beat per minute (bpm) in the first minute,  $p < 0.05$ ); the lowest value was observed in the 10th minute ( $396 \pm 10.3$  bpm,  $p < 0.001$ ). This value was lower by 14 % than the baseline value and it remained nearly constant till the end of the experiment.

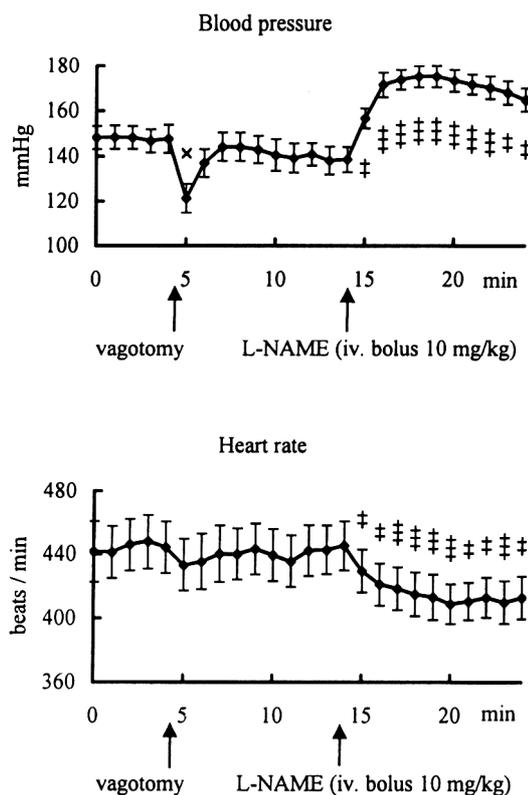


**Fig. 1.** Alteration of blood pressure and heart rate after administration of L-NAME (broken line) or physiological saline (solid line).  $\times$  values after L-NAME vs. baseline:  $\times p < 0.05$ ,  $\times \times p < 0.01$ ,  $\times \times \times p < 0.001$ .

#### Mechanism of the heart rate changes after L-NAME - Second series of experiments

The heart rate reached the minimum value 10 min after L-NAME administration and remained unaltered during the experiment (see above). Therefore, in the second series of experiments the blood pressure and heart rate values measured 10 min after L-NAME administration were used for statistical analysis. After vagotomy and/or metoprolol administration, ten minutes were allowed for stabilization, too.

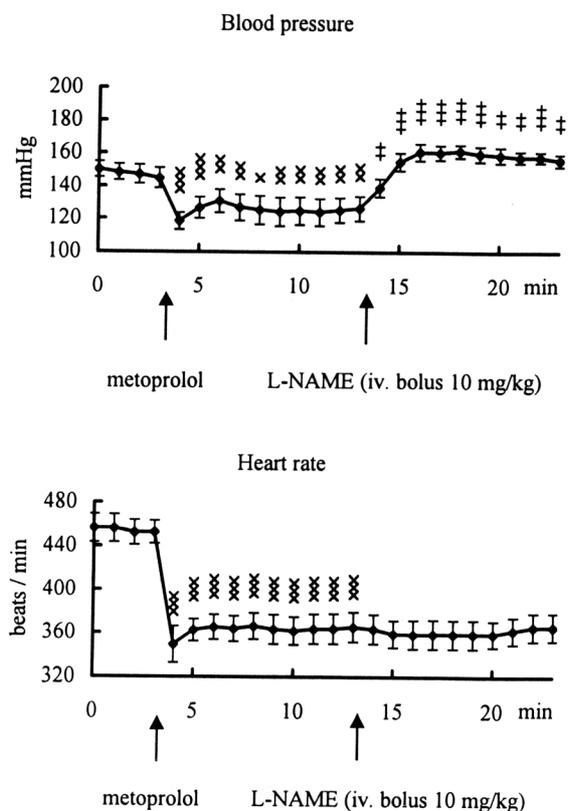
In the first group, the inhibition of nitric oxide synthesis increased arterial blood pressure (from  $142 \pm 8.1$  to  $161 \pm 6.0$  mm Hg,  $p < 0.05$ ) and decreased heart rate (from  $475 \pm 15.4$  to  $436 \pm 15.3$  bpm,  $p < 0.01$ ). Bilateral vagotomy performed 10 min after L-NAME administration failed to influence the bradycardia. Even ten minutes after vagotomy, heart rate was remained low ( $443 \pm 18.5$  bpm) and it did not differ from the value recorded before vagotomy ( $436 \pm 15.3$  bpm).



**Fig. 2.** Alteration of blood pressure and heart rate after vagotomy and after administration of L-NAME.  $\times$  values after vagotomy vs. baseline:  $\times p < 0.05$ .  $+$  values after L-NAME vs. 10th minute after vagotomy:  $++ p < 0.01$ ,  $+++ p < 0.001$ .

In the second group, bilateral vagotomy was performed after stabilization. Ten minutes after vagotomy, the blood pressure was nearly unchanged ( $139 \pm 5.5$  mm Hg) compared to the baseline ( $148 \pm 5.3$  mm Hg, Fig. 2). L-NAME significantly increased the blood pressure ( $165 \pm 5.1$  mm Hg,  $p < 0.01$ ). Vagotomy failed to influence the heart rate (baseline:  $444 \pm 16.3$ , after vagotomy:  $445 \pm 15.3$  bpm). L-NAME injection decreased the heart rate to  $413 \pm 13.3$  bpm ( $p < 0.01$ ). The percentage change of heart rate after L-NAME in vagotomized rats was similar to that seen in rats of the

first group without vagotomy ( $-7\pm 1.5$  and  $-8\pm 2.4$  %, respectively).



**Fig. 3.** Alteration of blood pressure and heart rate after metoprolol and after administration of L-NAME. × values after metoprolol vs. baseline: ×  $p < 0.05$ , ××  $p < 0.01$ , ×××  $p < 0.001$ . + values after L-NAME vs. 10th minute after metoprolol: ++  $p < 0.01$ , +++  $p < 0.001$ .

In the third group of animals, the injection of  $\beta_1$ -receptor antagonist metoprolol caused a significant drop in blood pressure (from  $145\pm 6.2$  to  $126\pm 7.5$  mm Hg,  $p < 0.01$ ), but L-NAME caused significant elevation (up to  $156\pm 3.6$  mm Hg,  $p < 0.01$ , Fig 3). The heart rate decreased from  $453\pm 10.5$  to  $366\pm 13.7$  bpm ( $p < 0.001$ ) after metoprolol administration and L-NAME failed to result in a further decrease (heart rate ten minutes after L-NAME:  $365\pm 12.8$  bpm).

In the fourth group the simultaneous application of vagotomy and metoprolol significantly decreased the blood pressure (from  $142\pm 5.11$  to  $120\pm 7.03$  mm Hg,  $p < 0.05$ ). Following L-NAME administration, blood pressure increased to  $146\pm 6.3$  mm Hg ( $p < 0.05$ ). The heart rate after vagotomy and metoprolol decreased from  $453\pm 14.1$  bpm to  $396\pm 13.6$  bpm ( $p < 0.01$ ). L-NAME treatment had no further effect on the bradycardia in this group ( $405\pm 11.9$  bpm).

## Discussion

In the present experiments, we investigated the effect of a single dose of L-NAME on the heart rate and the role of parasympathetic (vagal nerve) and sympathetic ( $\beta_1$ -receptor mediated) input to the heart in the L-NAME evoked bradycardia. A single intravenous injection of 10 mg/kg L-NAME resulted in an increase of arterial blood pressure for almost 20 min and a long-term decrease in the heart rate of anesthetized rats.

Bradycardia was observed after the blockade of nitric oxide production in several studies. The changes in the heart rate as function of time and the magnitude of the heart rate response after inhibition of nitric oxide synthesis strongly depends on the NOS inhibitor applied, its dosage, the way of administration and the species (Du *et al.* 1992, Jones and Brody 1992, Persson *et al.* 1992, Manning *et al.* 1993, Hennessy *et al.* 1994, Reid *et al.* 1994). As elevation of blood pressure was followed immediately by a decrease of heart rate in our experiments, a reflex bradycardia can be supposed. However, whilst the blood pressure returned to the control level in the 20th minute, the heart rate remained decreased till the end of the investigation. According to these results, two different phases of bradycardia can be distinguished: a) the immediate phase and b) sustained phase. The immediate phase could be caused by activation of baroreceptor reflex (elevation of blood pressure induces baroreceptor activation causing low heart rate) and in the sustained phase some other mechanisms may be involved.

To determine whether the cardiac innervation plays a role in the L-NAME evoked bradycardia, we first investigated the effect of parasympathetic denervation (vagotomy) and the effect of  $\beta_1$ -receptor antagonist metoprolol on the blood pressure and heart rate during nitric oxide synthesis blockade.

The magnitude of hypertension and bradycardia caused by L-NAME was the same both in vagotomized and non-vagotomized rats. Therefore, the bradycardia cannot be explained by the hypertension-induced increment of vagal tone. By contrast, others (Aisaka *et al.* 1989) have shown that atropine prevents the bradycardia caused by the nitric oxide synthesis blocker. The difference between their and our observations could be explained by species and drug difference (Aisaka and co-workers used L-N<sup>G</sup>-methyl-arginine and guinea pig). On the basis of other studies the heart rate effect of vagal nerve stimulation is not influenced by NOS inhibition (Sears *et al.* 1998) and the effect of nitric oxide on vagal

control of the heart seems to be depending on the species (Conlon and Kidd 1999).

In our experiments, the administration of metoprolol caused a distinct fall in blood pressure and heart rate. The blood pressure after L-NAME was elevated significantly, but no bradycardia was observed. This is in good agreement with the findings of Pegoraro and co-workers (Pegoraro *et al.* 1992), who reported that the L-NAME induced bradycardia was abolished by pretreatment of the non-selective  $\beta$ -receptor inhibitor propranolol. However, the elimination of the sympathetic innervation of the heart and adrenal catecholamine release markedly attenuated the L-NAME evoked bradycardia but could not completely prevent it (Jones and Brody 1992). The difference between these findings and the effect of receptor blockade raise the possibility that L-NAME might have a direct inhibitory effect on the  $\beta$ -receptors. This is supported by the observation that L-NAME attenuated the heart rate response to  $\beta$ -adrenergic stimulation (Reid *et al.* 1994). There might be an interference in intracellular mechanisms between cyclic AMP (intracellular messenger of  $\beta$ -receptors) and cyclic GMP (intracellular messenger of nitric oxide) (Klabunde *et al.* 1992).

As has been mentioned above, we can distinguish two periods of bradycardia. According to our results discussed above, the possible mechanism of bradycardia in the immediate period can be explained by the withdrawal of the sympathetic tone. The elimination of baroreceptor afferents can abolish the bradycardia evoked by L-NAME (Jones and Brody 1992), so the appearance of bradycardia immediately after the hypertension induced by nitric oxide inhibition could be explained by activation of baroreceptor reflex. However, the heart rate in our experiments remained low and unchanged even when the blood pressure had returned to

the control level. In the chronic experiments of Manning and co-workers (Manning *et al.* 1993), when nitric oxide synthase blocker was given to dogs for 11 days, the heart rate remained low after the termination of drug administration, although the blood pressure returned to the control level. This sustained bradycardia was not discussed in their study, and its mechanism has not yet been clarified. It was also found by Du and co-workers (Du *et al.* 1992) that a short time infusion of the NOS blocker results in an increase of blood pressure lasting 24 h and a decrease in heart rate for 48 h in conscious rabbits. These authors concluded that the sustained bradycardia couldn't be explained by a change of baroreceptor sensitivity. It is possible that inhibition of nitric oxide synthesis has a direct effect on the heart (Klabunde *et al.* 1992, Grocott *et al.* 1994, Hare *et al.* 1995) or that L-NAME affects on the central nervous system directly (Sakuma *et al.* 1992, Togashi *et al.* 1992, Jimbo *et al.* 1994). These effects could last longer than the effect on the peripheral vessels. This is supported by the fact that nitric oxide synthase is present in several central and peripheral sections of the sympathetic system (Anderson 1992, Anderson *et al.* 1993, Iadecola *et al.* 1993, Vicent and Kimura 1992).

In conclusion, our findings that metoprolol prevents the L-NAME induced bradycardia suggest that cardiac  $\beta_1$ -receptors play a crucial role in the development of bradycardia after L-NAME treatment in anesthetized rats.

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